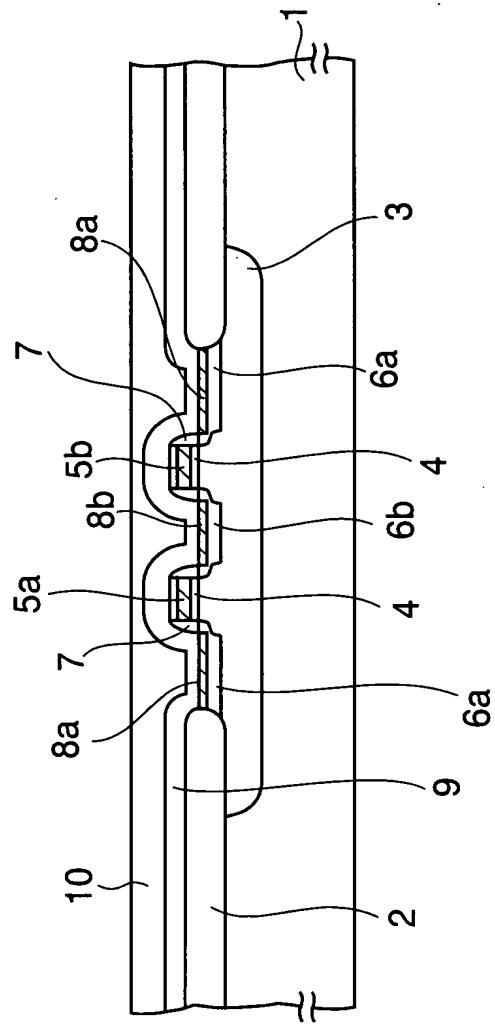


FIG. 1A



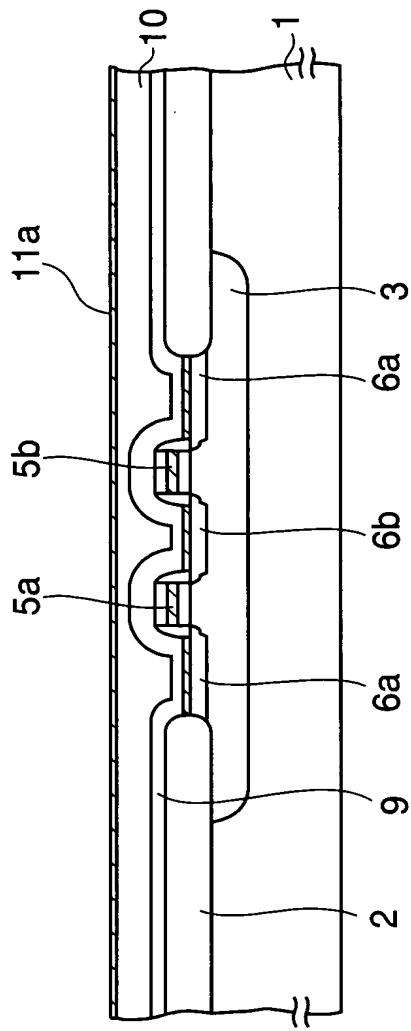


FIG. 1B

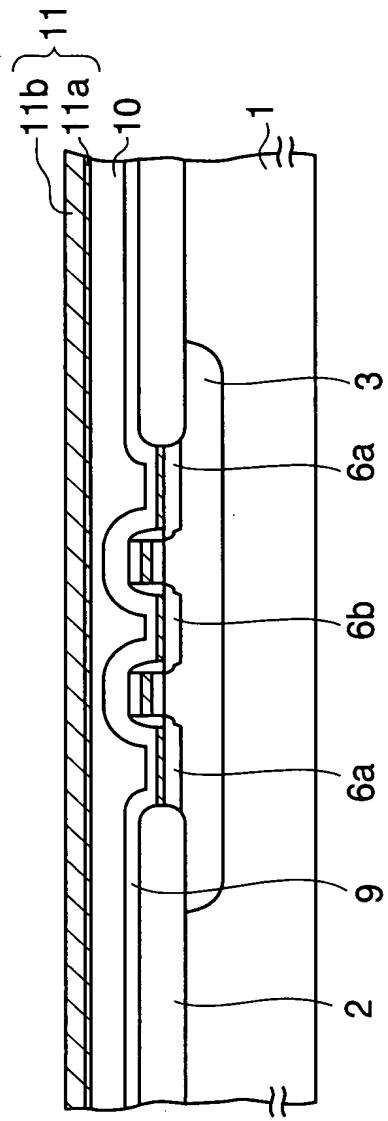
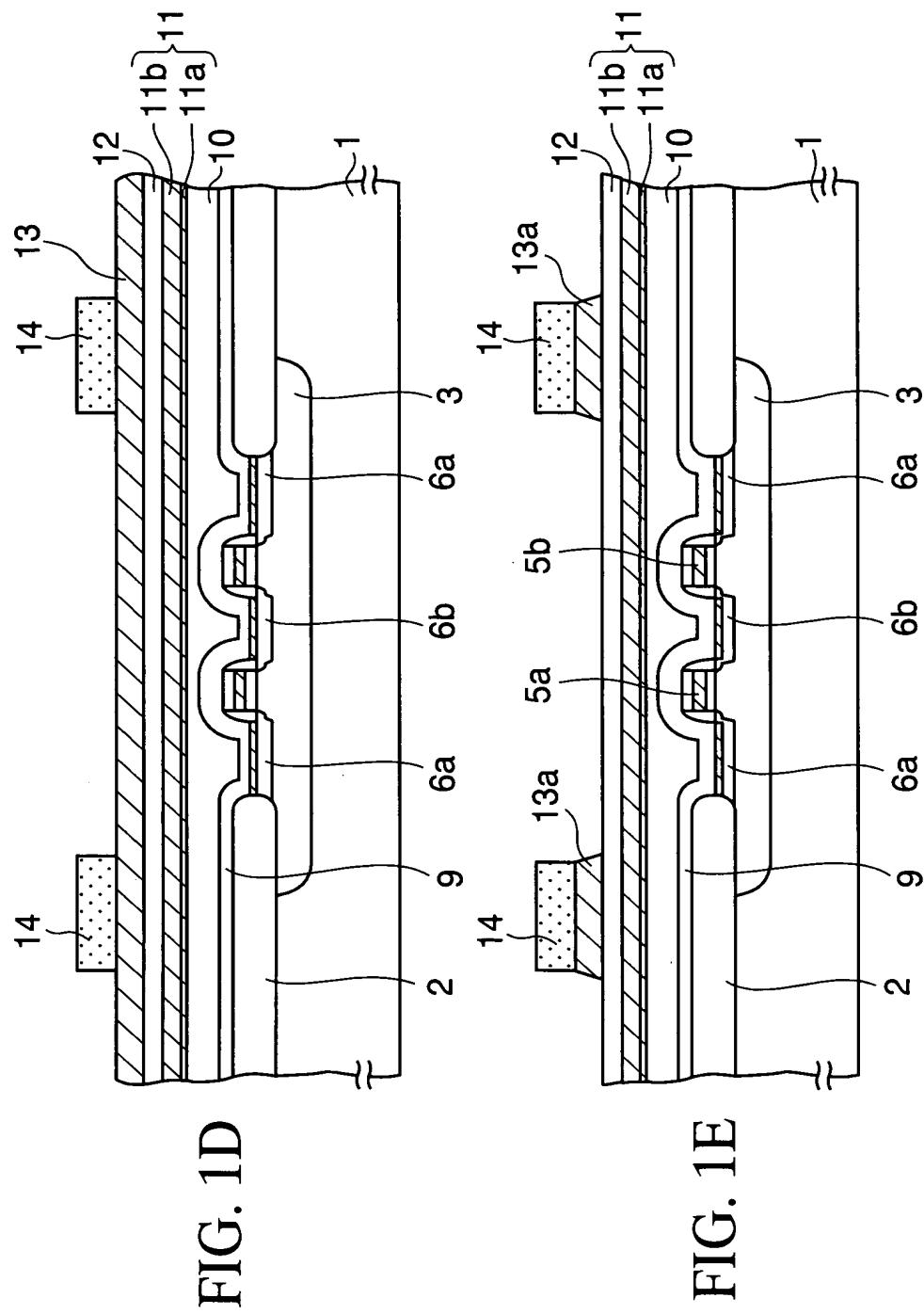


FIG. 1C



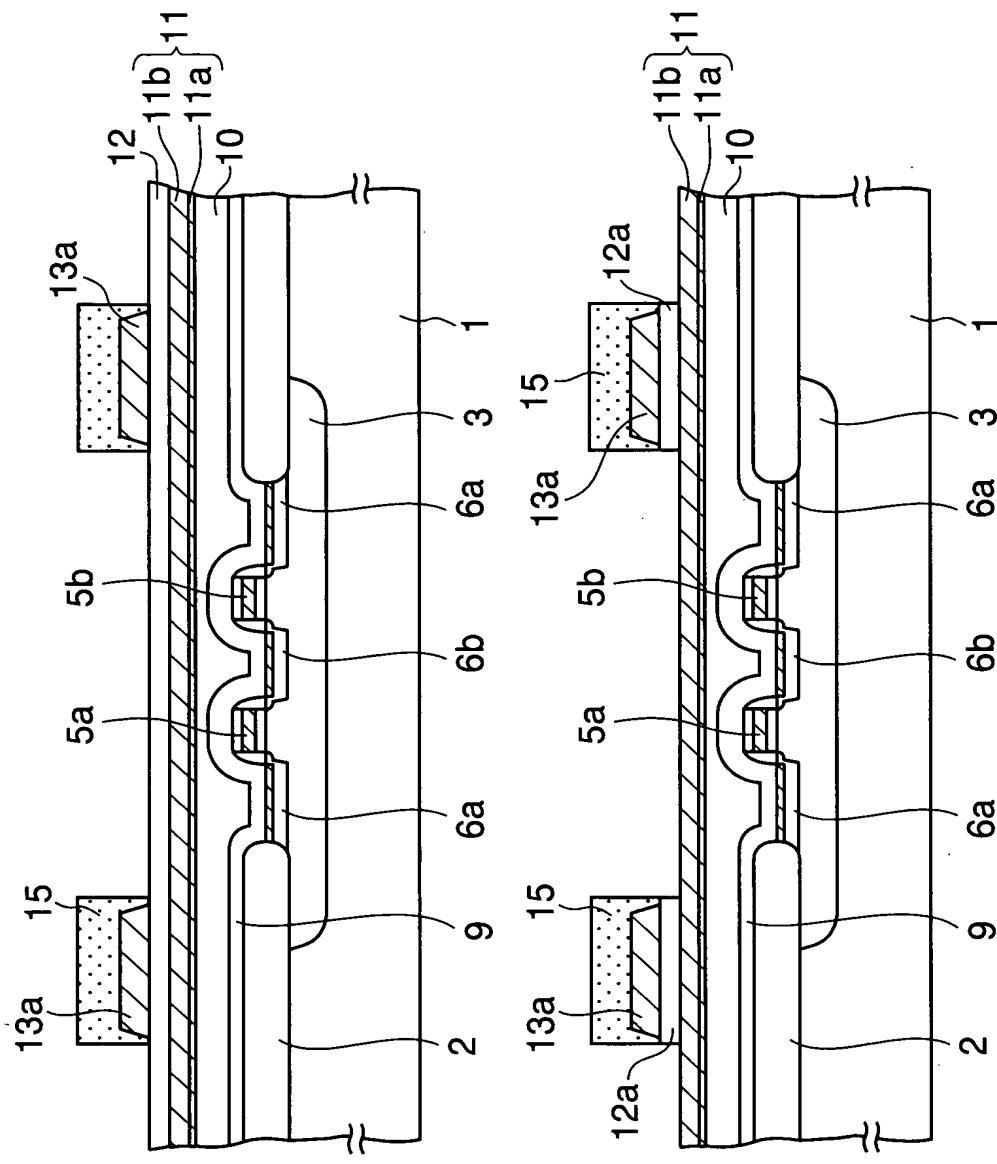


FIG. 1F

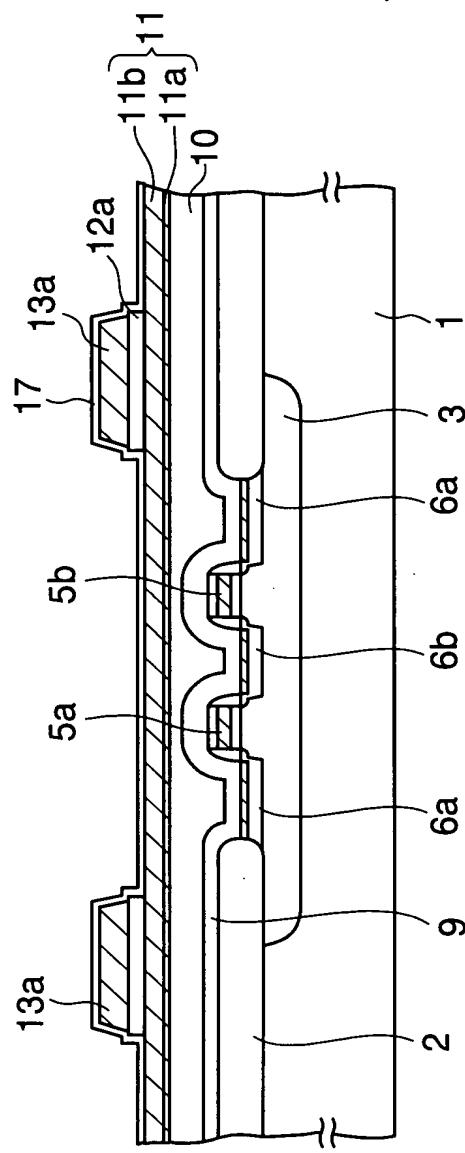


FIG. 1H

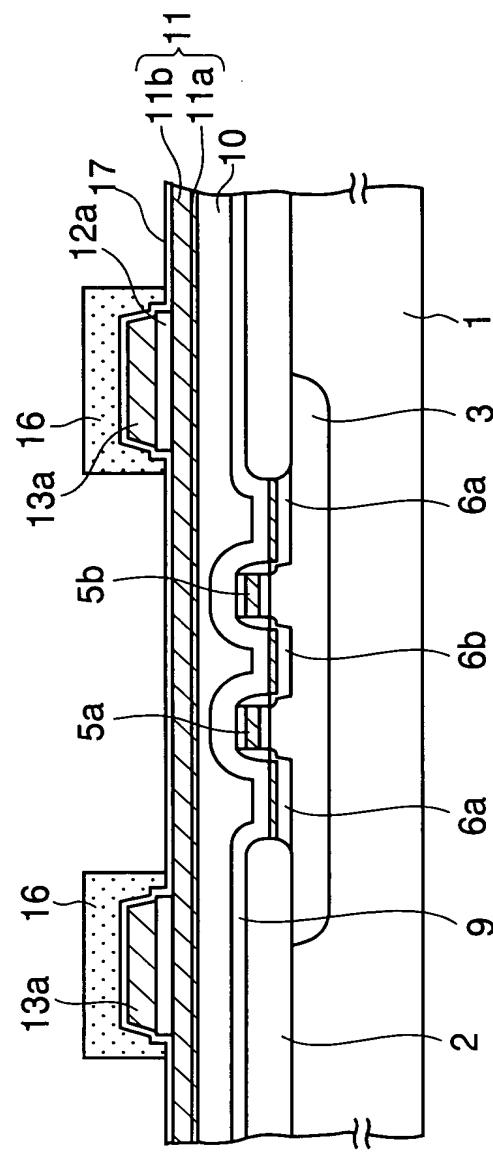


FIG. 1I

As an aqueous solution system to be employed for binding double-stranded nucleic acid to mineral supports, there may be used an aqueous solution containing from 1 to 5 M of guanidinium isothiocyanate, and/or from 1 to 8 M of guanidinium chloride, together with from 0.1 to 5% of sarcosinates, or from 5 mM to 200 mM of EDTA. For binding double-stranded nucleic acid, there may also be used a solution containing from 1 to 5 M of guanidinium thiocyanate, and/or from 1 to 8 M of guanidinium chloride, and from 5 mM to 200 mM of EDTA or EGTA.

BSPR:

3) Add 50 .mu.l of silica suspension (50% in lysis buffer) and incubate at room temperature for 10 minutes for the nucleic acids to bind, with repeated vortexing.

BSPR:

The isolated nucleic acids were analyzed on agarose gels stained with ethidium bromide. To this purpose, 1.2% formaldehyde or 1.2% 1 .times. TBE gels were prepared.

BSPR:

The examples described in the following will illustrate the performance of the process according to the invention. All nucleic acids accordingly isolated were electrophoretically analyzed and quantified by photometry. The OD<sub>260/280</sub> value was between 1.7 and 2.0 for all eluates.

DEPR:

Isolation of Whole Nucleic Acid

DEPR:

In the following reference examples 1 to 5, the binding, washing and elution conditions were respectively selected such that both DNA and RNA would bind to the mineral support and be eluted together.

DEPR:

Isolation of whole nucleic acid from kidney tissue

DEPR:

From 15 mg of kidney tissue (rat), whole nucleic acid was isolated according to standard protocol 4.1. The tissue was mixed with 400 .mu.l of L1 and homogenized, followed by addition of 280 .mu.l of B1. The first washing step was performed with W1, and the elution volume was 2.times.50 .mu.l.

DEPR:

Isolation of whole nucleic acid from liver tissue

DEPR:

From 7 mg of liver tissue (rat), whole nucleic acid was isolated according to standard protocol 4.1. The tissue was mixed with 300 .mu.l of L2 and homogenized, followed by addition of 200 .mu.l of B2. The first washing step was performed with W1, and the elution volume was 2.times.50 .mu.l.

DEPR:

Isolation of whole nucleic acid from HeLa cells

DEPR:

From 1.times.10<sup>sup.6</sup> HeLa cells, whole nucleic acid was isolated according to standard protocol 4.1. The cells were mixed with 400 .mu.l of L2 and homogenized, followed by addition of 200 .mu.l of B1. The first washing step was performed with W1, and the elution volume was 1.times.50 .mu.l.

DEPR:

Isolation of whole nucleic acid from plasma

DEPR:

Whole nucleic acid from plasma was isolated in two parallel runs according to

standard protocols 4.1 and 4.2, respectively. In each case, 800 .mu.l of L3 and 660 .mu.l of B2 were added to 200 .mu.l of plasma and mixed; homogenization was not necessary here. To the mixture for the "batch procedure" (4.2), there was additionally added 40 .mu.l of silica suspension. In both runs, the first washing step was performed with W2, and the elution volume was 2.times.100 .mu.l.

DEPR:

Fractional binding of RNA and DNA at constant GTC concentration and with increasing ethanol concentration

DEPR:

As shown in FIG. 1, the RNA fraction will bind to the mineral support under the conditions described already from ethanol concentrations of greater than 25% whereas the DNA fraction will bind only from ethanol concentrations of greater than 40%.

DEPR:

Isolation of Whole RNA

DEPR:

The examples illustrate the use of GTC, GuHCl or GTC/ethanol mixtures for the lysis of the starting materials. The integrity of the isolated RNA was verified by Northern Blotting or RT-PCR.

DEPR:

In these examples, the DNA not bound to the support was not further processed. The further purification of DNA from the column break-through will be shown in example 12. In addition, the DNA may be further purified by adjusting the binding conditions to those chosen in reference examples 1 to 5.

DEPR:

Isolation of whole RNA from spleen tissue

DEPR:

From 15 mg of spleen tissue (mouse), whole RNA was isolated according to standard protocol 4.1. The tissue was mixed with 350 .mu.l of L4 and homogenized, followed by addition of 350 .mu.l of B4. The first washing step was performed with W3, and the elution volume was 1.times.50 .mu.l.

DEPR:

Isolation of whole RNA from liver tissue (A)

DEPR:

Isolation of whole RNA from liver tissue (B)

DEPR:

From 15 mg of liver tissue (rat), whole RNA was isolated according to standard protocol 4.1. The tissue was mixed with 300 .mu.l of L6 and homogenized, followed by addition of 175 .mu.l of B1. The first washing step was performed with W4, and the elution volume was 1.times.50 .mu.l.

DEPR:

Isolation of whole RNA from HeLa cells

DEPR:

From 1.times.10.sup.7 HeLa cells, whole RNA was isolated in two parallel runs according to standard protocols 4.1 and 4.2, respectively. In each case, the cells were mixed with 350 .mu.l of L7 and homogenized, followed by addition of 350 .mu.l of B4. To the mixture for the "batch procedure" (4.2), there was additionally added 50 .mu.l of silica suspension. The first washing step was performed with W3, and the elution volume was 1.times.50 .mu.l.

DEPR:

Isolation of whole RNA from tobacco

DEPR:

For the isolation of whole RNA from plants, standard protocol 4.1 is slightly modified. After step 1) of the protocol (lysis), a centrifugation step at 5000 rpm in a table-top centrifuge is inserted to separate off unlysed cell components, such as fiber residues. The supernatant is removed, mixed with binding reagent and further processed according to the standard procedure from step 2).

DEPR:

From 100 mg of tobacco leaves, whole RNA was isolated according to standard protocol 4.1 as modified for plants. The powdered cell material was mixed with 600 .mu.l of L2 and homogenized, followed by addition of 350 .mu.l of B4. The first washing step was performed with W3, and the elution volume was 1.times.50 .mu.l.

DEPR:

Isolation of whole RNA from E. coli

DEPR:

For the isolation of whole RNA from bacteria, an additional step is inserted before performing the standard protocol in order to lyse the cell walls of the bacteria. The cell pellet is resuspended in 400 .mu.g/ml lysozyme in TE and incubated on ice for 5 min and at room temperature for 10 min. This is followed by lysing according to the standard procedure.

DEPR:

From 1.times.10.sup.9 E. coli cells, whole RNA was isolated according to standard protocol 4.1 as modified for bacteria. The pellet was resuspended in 80 .mu.l of 400 .mu.l/ml lysozyme in TE and incubated as described above. This was followed by addition of 270 .mu.l of L2, homogenization, and addition of 350 .mu.l of B4. The first washing step was performed with W3, and the elution volume was 2.times.50 .mu.l.

DEPR:

Isolation of DNA

DEPR:

In the following examples 9 and 10, the binding conditions were selected such that only DNA can bind to the mineral support whereas RNA will break through.

DEPR:

In these examples, the RNA not bound to the support was not further processed. The further purification of RNA from the column break-through will be shown in example 11. In addition, the RNA in the column break-through may be further purified by adjusting the binding conditions to those chosen in examples 2 to 8.

DEPR:

The selective DNA binding is performed in the lysis buffer in the absence of alcohol, i.e. step 2) of standard protocols 4.1 and 4.2 is omitted.

DEPR:

Isolation of genomic DNA from kidney tissue

DEPR:

Ten milligrams of kidney tissue (rat) was lysed in 700 .mu.l of L8. The DNA was bound to the mineral support without addition of binding reagent and washed with 700 .mu.l of L8 in the first washing step. Then, standard protocol 4.1 was performed from step 6). The elution volume was 2.times.50 .mu.l.

DEPR:

Isolation of genomic DNA from HeLa cells

DEPR:

1.times.10.sup.7 HeLa cells were lysed in 700 .mu.l of L9. The DNA was bound to the mineral support without addition of binding reagent and washed with 700 .mu.l of L9 in the first washing step. Then, standard protocol 4.1 was performed from step 6). The elution volume was 2.times.50 .mu.l.

DEPR:

The following examples 11 to 13 for the separated processing of RNA and DNA from the same cell lysate are combinations of the above examples for RNA, DNA or whole nucleic acid isolations.

DEPR:

Separation can be performed by either differential binding or fractional elution of RNA and DNA.

DEPR:

Separation of Whole RNA and Genomic DNA by Differential Binding

DEPR:

After the lysis, the conditions may be selected either such that DNA will first bind to the mineral support (example 11), or else RNA may be adsorbed in the first binding step while DNA is further processed from the break-through (example 12).

DEPR:

Isolation of genomic DNA and whole RNA from kidney tissue

DEPR:

Ten milligrams of kidney tissue (rat) was lysed in 350 .mu.l of L8, and the DNA was bound to the mineral support in the lysis buffer. To the column break-through, there was added 350 .mu.l of B4, and the whole RNA isolated in accordance with example 3.1. Isolation of the genomic DNA was performed as in reference example 1.

DEPR:

Isolation of whole RNA and genomic DNA from lung tissue

DEPR:

From 20 mg of lung tissue (rat), the whole RNA was isolated as described in example 2. The not bound genomic DNA in the column break-through was isolated by adding 350 .mu.l of B1 and 350 .mu.l of B5 and binding the DNA to the mineral support as described in standard protocol 4.1. The first washing step was performed with W1 and the elution volume was 2.times.50 .mu.l.

DEPR:

The binding conditions are selected such that the whole nucleic acid will bind to the mineral support. The DNA fraction is subsequently eluted while the RNA fraction remains bound. The eluted DNA is bound to another mineral support by readjusting to DNA binding conditions (cf. FIG. 1) and further processed.

DEPR:

Isolation of genomic DNA and whole RNA from liver tissue

DEPR:

Fifteen mg of liver tissue (swine) were lysed in 300 .mu.l of L2 according to standard protocol 4.1, 1) to 4), mixed with 250 .mu.l of B1, and the whole nucleic acid bound to the mineral support. The DNA fraction was eluted with 300 .mu.l of W5, while the support material with the still bound RNA fraction was treated according to standard protocol 4.1 from 5). The DNA fraction was isolated from the eluate by addition of 350 .mu.l of B1 and 250 .mu.l of B5 and binding to another mineral support according to standard protocol 4.1.

DETL:

TABLE 1 Washing buffer compositions for washing out DNA contaminations washing buffer sample 25 mM TRIS/HCl, % no. M GTC pH 7.5 ethanol 1 0.3 + 5 2 0.6 + 5 3 0.9 + 5 4 0.3 - 5 5 0.6 - 5 6 0.9 - 5 7-12 as in 1-6, but 10% EtOH 8-18 as in

1-6, but 20% EtOH R\*) 1.75 - 35 \*)This sample served as a reference; the washing buffer composition corresponded to the binding conditions.

CLPR:

6. The process according to claim 1, further comprising the step, whereby, prior to applying the sample to the first mineral support, cells in said source containing the nucleic acids are lysed with chaotropic substances present in concentrations of from 0.1 to 10 M.

CLPR:

19. The process according to claim 1, wherein said first and second mineral supports are porous or non-porous and comprised of metal oxides or mixed metal oxides, silica gel, glass particles, powdered glass, quartz, alumina, zeolites, titanium dioxide, or zirconium dioxide, the particle size of the mineral supports is from 0.1 .mu.m to 1000 .mu.m, and the pore size of porous mineral supports is from 2 to 1000 .mu.m.

CLPR:

21. The process according to claim 1, wherein the single- or double-stranded nucleic acids obtained, thereby are respectively purified by chromatographic steps.

CLPV:

a) applying to a first mineral support an aqueous solution containing a sample of said source under conditions whereby said first mineral support adsorbs only one of said single- or double-stranded nucleic acids followed by, optionally, washing said first mineral support; and

CLPV:

b) applying to a second mineral support the other of said single- or double-stranded nucleic acids, which was not adsorbed by the first mineral support, in an aqueous solution containing materials with alcohol groups.

CLPV:

i) the applying step to the first mineral support comprises adding to said aqueous solution salts and materials carrying alcohol groups in amounts such that the single-stranded, but not the double stranded, nucleic acids are adsorbed on the first mineral support, followed by, optionally, washing said first mineral support,

CLPV:

ii) the double-stranded nucleic acids, which were not adsorbed on the first mineral support, are applied to the second mineral support in the presence of materials with alcohol groups in amounts such that the double-stranded nucleic acids are adsorbed on the second mineral support, followed by, optionally, washing said second mineral support, and

CLPV:

i) the applying step to the first mineral support comprises adding said aqueous solution with materials which complex alkaline-earth metal ions, in the absence of materials with alcohol groups, such that double-stranded, but not single-stranded nucleic acids are absorbed on the first mineral support,

CLPV:

ii) the single-stranded nucleic acids, which were not absorbed on the first mineral support, are applied to the second mineral support in the presence of materials with alcohol groups in amounts such that the single-stranded nucleic acids are absorbed on the second mineral support, followed by optionally, washing said second mineral support, and

CLPV:

i) the applying step to the first mineral support comprises adding to said aqueous solution wetting, washing, or dispersing agents, in the absence of materials with alcohol groups, such that said double-stranded nucleic acids are absorbed on the first mineral support, followed by, washing said first

mineral support,

CLPV:

ii) the single-stranded nucleic acids, which were not absorbed on the first mineral support, are applied to the second mineral support in the presence of materials with alcohol groups in amounts such that the single-stranded nucleic acids are absorbed on the second mineral support, followed by optionally, washing said second mineral support, and

CLPV:

i) the applying step to the first mineral support comprises adding to said aqueous solution salts and materials with alcohol groups in amounts such that both the single-stranded and double-stranded nucleic acids are adsorbed on the first mineral support,

CLPV:

iii) the one of the single- or double-stranded nucleic acids, which was first eluted from the first mineral support, is applied to the second mineral support under conditions whereby the nucleic acids first eluted from the first mineral support are adsorbed on the second mineral support, followed by eluting the nucleic acids from the second mineral support.

FIG. 3

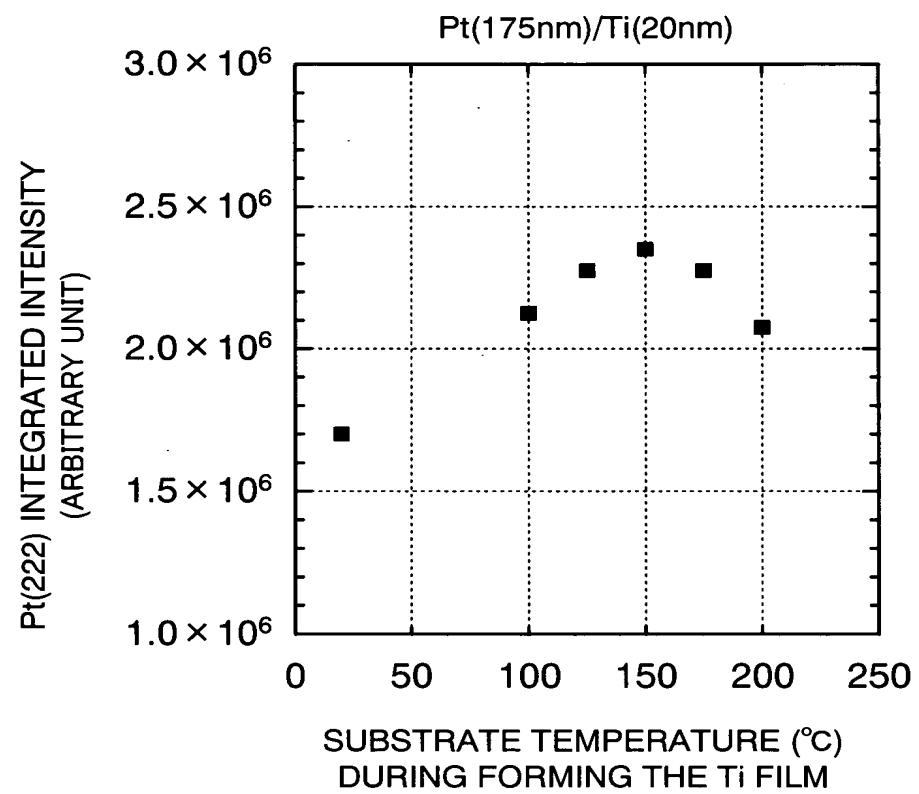


FIG. 4

